Metabolic Engineering of Light and Dark Biochemical Pathways in Wild-Type and Mutant *Synechocystis* PCC 6803 Strains for Maximal, 24-Hour Production of Hydrogen Gas

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This talk will describe results from an ongoing GTL project in which we are using the cyanobacterial species *Synechocystis* PCC 6803 to address two main factors affecting H2 production in PCC 6803: NADPH availability and O2 sensitivity. H2 production in PCC 6803 requires that the NADP pool be highly reduced, which can be problematic because several metabolic pathways potentially can act to raise or lower NADPH levels. Also, the [NiFe]-hydrogenase (H2ase) in PCC 6803 is reversibly inactivated at very low O2 levels due to binding of O2 at the active site. Largely because of this O2 sensitivity and the requirement for high NADPH levels, much of the overall H2 production occurs under anoxic conditions in the dark, supported by breakdown of glycogen or other organic substrates accumulated during photosynthesis. Also, other factors, such as N or S limitation, pH changes, presence of other substances, or deletion of particular respiratory components, can affect light or dark H2 production. Therefore, we have used H2 production profiling and metabolic flux analysis to examine light and dark H2 production under a number of culture conditions with wild-type (WT) PCC 6803 cells and with mutant strains. Also, some of the mutants we have created have shown themselves capable of increased H2 production.